Development of nucleated red blood cells separation method using micro flow channel

1. Introduction Conventional prenatal fetal DNA diagnosis methods are invasive when fetal cells are recovered, and it has a constant risk of miscarriage of the fetuses. For example, amniocentesis has a risk of miscarriage and infection with 0.2~0.3 % rate. Even though, prenatal fetal diagnosis is implemented 5 million times per year in the world. It should be non-invasive to fetus. Recently, the researches for non-invasive prenatal diagnosis using maternal peripheral blood were studied. In the maternal peripheral blood, nucleated red blood cells (NRBCs) that have migrated from the fetus exist and it is possible to analyze the retained DNA in them. However, the concentration of NRBCs in the maternal blood is very rare for example, 1~2 cells/mL, so that the separation of them is very difficult. Takabayashi et.al., reported that NRBCs were recovered successfully from the maternal blood by combination of density gradient centrifugation and image processing, and have also been successful in gender diagnosis. However, the method took too long time of 20 hours to recover the NRBCs and is inefficient for practical use. To overcome this problem, further concentration of NRBCs between the density gradient centrifugation and the image processing was effective because it reduces the number of blood cells to be searched over. To do this, we focused on the size and hardness of blood cell. Filters and chromatography also can separate blood cells, but it is difficult to recover trapped blood cells. Here, we developed micro-fluidic chip with separation/concentration and recovery mechanism by applying semiconductor micro-fabrication technology. In order to shorten the time for the separation and concentration of NRBCs by chip, we tried to develop multiple micro-gap chip. In addition, we develop fully automated processing equipment aiming at improvement of reproducibility and convenience. From those, the efficiency for the recovery of NRBCs was improved very much, which contributes to realize noninvasive prenatal genetic diagnosis.

2. Experimental The design of micro-gap chip was done using Adobe Photo Illustrator. Micro-gap chip is fabricated by photolithography. The height of the gap of the micro-gap chip was made at 1.2 μm, 1.5 μm, and 1.9 μm. As preparation, maternal blood was centrifuged by density gradient. Maternal blood was delivered to the micro-gap chip, with the flow rate to the chip of 1 μL/min, and the process time for separation and concentration of 30 minutes. Red blood cell, white blood cell count was measured using FACS. In order to speed up nucleated red blood cell separation by chip, multiple micro-gap chip was designed with Adobe Photo Illustrator and fabricated using photolithography. The number of micro-gap channels was increased 5 times from 90 to 448. Umbilical cord blood diluted 10 times was delivered to the chip. Here the flow rate to the chip was 11 μL/min, 22 μL/min, 33 μL/min. PLC (programmable logic controller) was used for automation of multiple micro-gap chip, and flow operation and diaphragm operation were carried out by air pressure control using an actuator and an air cylinder.

3. Results and discussion The blood cell can be trapped at the interstitial spaces of the micro-gap chip, and the trapped blood cells can be recovered by opening the membrane gap. NRBCs were discovered from the recovered solution. When the height of the micro-gap channel was 1.0 μm, the collection rate of NRBCs was 92%. When the micro-gap channel height was 1.5 μm and 1.9 μm, the collection rate of NRBCs was 75%, 25%. Removal rates of red blood cells and white blood cells were 93% and 98%. The processing time for one specimen was 90 minutes. The collection rate of NRBCs was 84% at the flow rate of 11 μL / min in multiple micro-gap chip. The collection rate was 66% when the flow rate was 22 μL / min and 60% when the flow rate was 33 μL / min. Removal rates of white blood cells and red blood cells were 98% and 84% at the flow rate of 11 μL/min. Those were 93% and 99% when the flow rate was 22 μL/min, and 92% and 94% when the flow rate was 33 μL/min, respectively. From these results, the time for automatic image processing can be expected to be shortened from 20 hours to 1 hour. Moreover, the number of tests can be increased from 1 sample per day to 20 samples per day. By pressure control using PLC, flow operation and membrane control were automated. The automation of multiple micro-gap chip was achieved from sample introduction to recovery.

4. Conclusion The fabricated micro-gap chip was able to trapped NRBCs. When the height of the micro-gap channel was 1.0 μm and the flow rate was 1.0 μL/min, the collection rate of NRBCs was 92%. Removal rates of red blood cells and white blood cells were 93% and 98%, respectively. In the multiple micro-gap chip, the collection rate of NRBCs was 84% at flow rate of 11 μL/min. Here, the removal rates of red blood cells and white blood cells were 84% and 98%. Using this chip, the total throughput of the recovery of NRBCs is expected to be improved by factor 20. The automation of micro gap chip operation is also achieved for convenience. From these, it is expected that the developed micro-gap chip will greatly contribute to non-invasive prenatal diagnosis.

Keyword: Noninvasive diagnosis, NRBCs, Cell hard and size, Micro- fluidic-chip, Micro gap.